The role of the *Caenorhabditis elegans* anterior *Hox* gene *ceh-13* in controlling cell migration and fusion

Ph.D. Theses

Tihanyi Borbála

Eötvös Loránd University, Faculty of Sciences
Biology Ph.D. School
Classical and Molecular Genetics PhD Program

Head of PhD School: Prof. Anna Erdei, member of HAS
Head of PhD Program: Prof László Orosz, member of HAS
Supervisor: Tibor Vellai Ph.D. associate professor

Eötvös Loránd University, Faculty of Sciences
Department of Genetics

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Introduction and aims

Hox genes encode evolutionary conserved transcriptional factors which control cell fates along the anteroposterior axis during animal development. They are characterized by clustering at a single genomic site and colinearity between their domains of function and genomic map position along the body axis of the embryo. The homologous clusters of *Hox* genes can be found in all bilaterians and they play similar roles in controlling animal patterning. In humans, the malfunction of *Hox* genes can result in serious morphological abnormalities and early lethality. I have studied the role of the anterior and middle paralog *Hox* genes in cell migration and cell fusion as well as their interactions with each other in controlling these developmental processes using the soil nematode *Caenorhabditis elegans* as a genetic model organism.

The *C. elegans Hox* cluster consists of 6 genes which represent 4 canonical *Hox* homologous groups: the anterior homolog, *ceh-13*, the central homolog *lin-39* and *mab-5*, and the posterior homolog genes *egl-5*, *nob-1* and *php-3*. The *C. elegans Hox* cluster differs in some characteristics from its counterparts in other organisms in that the anterior ortholog, *ceh-13* is positioned downstream of the medial-group gene *lin-39* on the chromosome due to an inversion. The functional consequence of this unusual genomic organisation remains unknown. Essential embryogenesis and viability requires only the anterior paralog *ceh-13* and the posterior paralog *nob-1*. The other members of the nematode *Hox* cluster function during postembryonic development, only.

The developmental function of the anterior ortholog *ceh-13* is the less known among the *C. elegans Hox* genes. The absence of *ceh-13* activity results in embryonic lethality (mutants exhibit serious morphological abnormalities), yet a small percent of the *ceh-13(-)* mutants are able to develop into fertile adults. Furthermore, the embryonic expression of *ceh-13* is not restricted to the anterior body region but overlaps with the expression domains of other *Hox* paralogs, especially with that of the central homolog *lin-39* and *mab-5*. These data raise the possibility that there might be a genetic interaction (potential functional redundancy) between *ceh-13* and the middle-group *Hox* paralogs. However, the interaction of *ceh-13* with other members of the *C. elegans Hox* cluster is not known. There is no information about the role of *ceh-13* during postembryonic development, either.

During my PhD work I have studied the genetic interactions of *ceh-13* with other

members of the *C. elegans Hox* cluster, especially with the medial homolog genes *lin-39* and *mab-5*. Furthermore, I have investigated the role of *ceh-13* in postembryonic developmental processes which are known to be controlled by *lin-39* and *mab-5*. I have also examined the sequencial similarity and phylogenetic relation of the *C. elegans Hox* paralogs using bioinformatic tools.

I was also involved in a project which focused on studying the transcriptional regulation of the medial homolog *Hox* gene *lin-39* during hermafrodite vulva development. Within this project, I have investigated the genetic interactions of *tra-1* (the terminal regulator of the nematode sex determination gene cascade) and the *synMuv* (*synthetic Multivulva*) genes. These genes are known to control the expression of *lin-39* which acts as the central regulator of vulva development.

Methods

Expression analysis

I used translational fusional HOX::GFP riporter constructs to study and compare the expression patterns of the *C. elegans Hox* paralogs. Transgenic strains carrying *ceh-13::gfp*, LIN-39::GFP, MAB-5::GFP or EGL-5::GFP constructs were kindly provided by Dr Vellai Tibor, Prof. Alex Hajnal, Prof. Cynthia Kenyon and Henrique B. Ferreira. I have constructed translational PHP-3::GFP and NOB-1::GFP riporter constructs, which were transformed by microinjection and integrated by UV irradiation on order to create stable transgenic worms. Transgenic strains were back-crossed at least eight times.

I have studied the expression of *Hox* genes in *comma stage* and *two fold stage* embryos and in L1-L2 stage larvae.

Mutant analysis and rescue experiments

For mutant analyses, mutant strains were obtained from the CGC (*Caenorhabditis elegans*

Genetics Center). To study genetic interactions between *ceh-13, lin-39* and *mab-5* and between *tra-1* and the *synMuv* genes, I created douple and triple mutant strains and examined the mutant phenotypes exhibited by these strains. For the *Hox* rescue experiments, I introduced a translational LIN-39::GFP riporter construct (the extra copy of *lin-39*) as well as the *mab-5(e1751gf)* mutation into *ceh-13(sw1)* null mutant genetic background to study their effect on the survival and developmental abnormalities of *ceh-13(-)* mutant animals.

RNA interference

In some experiments, the mutant phenotypes caused by the inactivation of certain *Hox* genes made it impossible to use genetic mutations. In such cases, I used RNAi (feeding method) to reduce gene function. dsRNA specific to the mRNA of the gene of interest was procuced by a bacterial strain (HT115) previously transformed by the recombinant vector pPD129.36 carrying the sequence of the gene to be inactivated. Inducible RNAi agar plates were seeded with the specific HT115 bacterial strain described above, then 3-5 L3 stage larvae were transferred onto the plates. The phenotype of F1 progeny was observed. RNAi experiments were carried out at 25 ºC.

Cell migration and cell fusion

To study the role of the anterior and middle paralog *Hox* genes in cell migration I used MEC-7::GFP and TAX-4::GFP riporter constructs which are expressed in different neuronal cell lineages. By crossing, I created transgenic strains carrying MEC-7::GFP or TAX-4::GFP constructs in single and double *Hox(-)* mutant genetic backgrounds. In these strains, I scored the number, position and the axongrowth of different neurons, as compared to wild type.

To examine the effect of *Hox* genes on the fusion of epidermal cell, I used a AJM-1::GFP riporter to visualize cell boundaries. I examined the number and the fusion pattern of P ectodermal blast cells, Pn.p daughters and their descendant that make up the hermafrodite vulva tissue, and also that of the V and seam cells in single and double *Hox(-)* mutant strains versus to wild-type background.

Phylogenetic analysis

For clustering the *C. elegans Hox* genes we applied the Bayesian phylogenetic method and used the 177 nucleotide long homedomain part of sequences. To calculate the tree, we used MrBayes v3.1.2 software. Aminoacid sequences of the *C. elegans* HOX proteins were also aligned by using the ClustalW software (available at the European Bioinformatics Institute's website: http://www.ebi.ac.uk/Tools/clustalw2/index.html).

Results

Characterization of the pleiotropic Ceh-13(-) mutant phenotype and *ceh-13* expression pattern:

- *ceh-13* affects the morphogenesis of the anterior, middle and posterior body regions of the animal
- several aspects of the pleiotropic Ceh-13(-) mutant phenotype resemble to the phenotypic defects caused by the inactivation of other *Hox* paralogs
- in embryos and early (L1-L2) stage larvae *ceh-13* is expressed all along the anteroposterior body axis; in certain cell types ceh-13 expression persists during adulthood as well
- the embryonic expression of *ceh-13* overlaps with the expressional domain of all other members of the *C. elegans Hox* cluster; in later developmental stages there is an overlap between the expressional domains of *ceh-13, lin-39* and *mab-5*

Genetic interaction of *ceh-13* with *lin-39* and *mab-5*:

- simultaneous inactivation of *ceh-13* and *lin-39* or *ceh-13* and *mab-5* results in a synthetic phenotype which differs from the phenotype of the corresponding single mutants

- *ceh-13(sw1)lin-39(RNSi)* and *lin-39(n1760)ceh-13(RNSi)* animals exhibit a fully penetrant (100%) embryonic lethality, while *mab-5(e1239)ceh-13(RNSi)* animals a 94% penetrant embyonic lethal phenotype – these mutant phenotypes are more severe and occur with a much higher penetrance than in either of the single mutants or RNSi worms
- in *ceh-13(sw1)mab-5(e1751gf)* double mutant and *ceh-13(sw1)*LIN-39::GFP worms embryonic or early larval lethality is less penetarnt and the percent of escapers which are able to develop to adulthood is much higher, as compared to *ceh-13(sw1)* single mutants
- extra copies of *lin-39* or a gain-of-function mutation of *mab-5* is able to suppress the morphological abnormalities, small body size, slow growth and movement defects caused by *ceh-13* deficiency [in *ceh-13(sw1)mab-5(e1751gf)* double mutant and *ceh-13(sw1)*LIN-39::GFP animals]

The role of *ceh-13* in postembryonic developmental processes controlled by *lin-39* and *mab-5*:

- in *ceh-13(-)* mutants the position of the Q descendant neurons is altered both at the anterior and posterior body parts
- inactivation of *ceh-13* results in the mispositioning and axonal outgrowth defects of the ALM and PLM neurons, and also of cells marked by a TAX-4::GFP neuronal fate marker
- *ceh-13(sw1)* mutant hermafrodites exhibit an abnormal fusion pattern of the Pn.p epidermal cells at both anterior and posterior body regions
- *ceh-13(sw1)* mutation causes defects in the fusion pattern and adhesion of the P ectodermal precursor cell, V and seam-cells
- in *ceh-13(sw1)mab-5(e1751gf)* double mutants, a gain-of-function mutation of *mab-5* is able to suppress the cell positioning and cell fusion defects caused by *ceh-13* deficiency
- *ceh-13(sw1)* mutant hermafrodites show various vulva mutant phenotypes (including Vulvaless, Protruded vulval morphology, Multivulva); an abnormal (asymmetric) development of the vulval structure can also be observed using an AJM-1::GFP

riporter

- *ceh-13(sw1)* mutation reduces the average number of induced vulval cells in *synMuv AB* double mutant genetic backgrounds, in which vulval induction is overactivated

Sequencial similarity and phylogeny of the *C. elegans Hox* genes:

- multiple/pair-wise sequence alignment reveals higher similarity among the genes *ceh-13, lin-39* and *mab-5*, as well as among *egl-5, nob-1* and *php-3* than between the two groups
- phylograms generated by clustering the *C. elegans Hox* genes show that the middle *Hox* paralogs *lin-39* and *mab-5* are more closely related to the anterior gene *ceh-13* than to the posterior paralogs *egl-5, nob-1* and *php-3*

Genetic interaction of *tra-1* and the *synMuv* genes in regulating vulva development:

tra-1 encodes the terminal transcription factor of the nematode sex-determination pathway. Vulva is a hermaphrodite-specific tissue, the development of which is negatively regulated by the (redundant) *synMuv* pathways.

- *tra-1(e1099)* mutation or *tra-1* RNSi treatment increases the average vulva number in *synMuv AB* double mutant genetic backgrounds
- *fem-3* RNSi (the inactivation of *fem-3* causes the hiperactivation of *tra-1*) results in the reduction of the average vulva number in *synMuv A(-) B(-)* double mutants
- inactivation of *tra-1* results in a Muv phenotype in *synMuv A(-)* mutant genetic background

Conclusions

I have studied the role of the C. elegans anterior *Hox* gene *ceh-13* in postembryonic developmental processes as well as its genetic interaction with other members of the *C.*

elegans Hox cluster, especially with the middle *Hox* paralogs *lin-39* and *mab-5*.

By phenotypic analysis, I showed that the inactivation of the anterior ortholog, *ceh-13* causes morphological abnormalities not only at the anterior but also at the middle and posterior body parts of the mutant animals. These defects resemble to those observed in other *Hox(-)* mutants. I constructed specific translational fusion HOX::GFP riporter constructs to compare the expression patterns of *C. elegans Hox* paralogs. This analysis revealed that the embryonic expression of *ceh-13* overlaps with the expressional domains of all the other members of the *C. elegans Hox* cluster. In larval stages the expression domain of *ceh-13* overlaps with that of *lin-39* and *mab-5*. These data together with the results of the *Hox* phenotype analysis suggest that *ceh-13* affects cell fates not only at the anterior but also at the middle and posterior body parts. Thus, *ceh-13* represents an exception from the rule of colinearity.

I showed that *ceh-13* genetically interacts with *lin-39* and *mab-5* in controlling embryonic development. Furthermore, extra copies of *lin-39* and a gain-of-function mutation of *mab-5* are able to suppress the developmental defects caused by *ceh-13* deficiency. These results suggest a partial functional redundancy between these *Hox* genes. LIN-39 and MAB-5 proteins are able to substitute CEH-13 function in controlling morphogenesis and viability. This may explain the presence of *ceh-13(-)* null mutant escapers in the population.

I also studied the role of *ceh-13* in postembryonic developmental processes which are known to be controlled by *lin-39* and *mab-5*. I found that *ceh-13* influences the migration of Q neuronal descendants and the fusion of Pn.p epidermal cells with the hypodermis. *ceh-13* also affects the hermafrodite vulva development. The effect of *ceh-13* on cell fate specification is obvious in both anterior and mid-posterior pody regions where its functional domain overlaps with that of *lin-39* and *mab-5*.

Data from multiple sequence alignment (using bioinformatic tools) revealed that the closest paralogs of *ceh-13* within the *C. elegans Hox* cluster are *lin-39* and *mab-5*.

The *Hox* clusters of bilaterians arose by the sequencial tandem duplications of an ancient „*ProtoHox*” ancestor. An early gene duplication event of this „*ProtoHox*” gene might have given rise to the ancestors of the anterior and posterior paralogous groups. The sequence similarity and partial functional redundancy among *ceh-13, lin-39* and *mab-5* support our hypothesis that the middle *Hox* paralogs evolved from an anterior ancestor during the evolution of the *Hox* clusters. Together, my findings may help to understand better how *C. elegans Hox* genes function during development and how they may have emerged during evolution.

Publications

Szabó E, Hargitai B, Regős Á, **Tihanyi B,** Barna J, Borsos É, Takács-Vellai K, Vellai T (2009). TRA-1/GLI controls the expression of the Hox gene *lin-39* during *C. elegans* vulval development. *Dev. Biol.* 330(2): 339-348.

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